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Studies on the Plasma Membrane of Normal and Psoriatic Keratinocytes. 6. Cell Surface and Shed Glycoproteins

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Quantification and characterization of [^{3}H]-fucose-labeled cell surface glycoproteins are reported. Two approaches have been compared; first the analysis of glycoprotein shed spontaneously into the medium during incubation of keratinocytes in vitro, and second the study of material released by exposure of the cell to proteolytic enzymes. It is shown that psoriatic keratinocytes "shed" glycoprotein more rapidly than normal, although the material is of similar molecular weight (mainly "biantennary" transferrin type glycopeptides). By contrast, the percentage of glycoprotein released by proteolysis of psoriatic keratinocytes is normal, but the molecular weight distribution of the labeled glycopeptides is markedly altered. The abnormal turnover and composition of fucose-labeled glycoproteins from the cell surface may be related to the loss of growth control in psoriatic epidermis.

Cell surface glycoproteins play an important role in growth regulation [1,2]. Loss of growth control (e.g., following transformation) appears to be accompanied by alterations in the carbohydrate moieties of glycoproteins [3]. These changes are not confined to the cell surface, but are seen also in glycoproteins of internal membranes [1,4]. Most of the above-mentioned studies on glycoproteins have been carried out with fibroblasts. More recently, cell surface glycoconjugates of epidermal origin have been investigated [5–7] and it has been shown [8] that the incorporation of D-[^{3}H]-glucosamine into extracellular glycosaminoglycans and proteoglycans by psoriatic epidermis is greatly diminished compared to normal.

In previous publications from this laboratory [9–12] striking abnormalities have been reported in the metabolism and composition of fucose-labeled glycoproteins derived from psoriatic keratinocytes. The possibility of these findings resulting from an increased growth fraction in the psoriatic lesion has been ruled out [12]. In order to establish whether the above changes are also reflected in the plasma membrane, the cell surface glycoconjugates of psoriatic and normal keratinocytes have been investigated. In addition, the turnover of cell surface glycoproteins has been studied by characterization and quantification of shed glycoproteins.

MATERIALS AND METHODS

Materials

Sephadex G-100 was obtained from Pharmacia (Uppsala, Sweden). All other chemicals were as described previously [11,12]. The selection of psoriatic patients and healthy controls was also as previously reported [13].

General Methods

The methods that have been used here are identical to those reported previously [11,12] except for 2 new procedures: namely, the isolation of shed glycoconjugates and the proteolytic enzyme treatment of keratinocytes (see below). Briefly, keratotome slices were taken from healthy controls, uninvolved psoriatic skin, and psoriatic lesions. Keratinocytes were isolated by trypsinization in the presence of dithioerythritol. The washed keratinocytes were suspended in TC199 medium supplemented with 20% calf serum and were labeled for 24 hr with [^{3}H]-fucose. After centrifugation the pellet was further processed as described under "Proteolytic Enzyme Treatment of Keratinocytes." The remaining supernatant was used for determination of shed glycoconjugates.

Shedding of Labeled Glycoconjugates

The shedding of labeled material into media was determined using 4 alternative procedures. In all experiments the nonspecific binding of labeled sugar to components of the media was determined by the omission of cells.

1. Direct trichloroacetic acid (TCA) precipitation: To 0.5 ml of the incubation medium, 0.5 ml of ice-cold 10% TCA (w/v) was added. The pellet obtained after centrifugation was washed 3 times with 1-ml portions of 5% TCA (w/v) and counted.

2. Papain treatment followed by Sephadex G-50 chromatography: Incubated medium (0.5 ml), buffered with 0.2 ml 0.2 M acetate buffer (pH 6.5) and containing cysteine (2 mg/ml), was incubated with 0.1 ml papain (3 mg) for 24 hr at 60°C. After cooling and centrifugation the resulting supernatant was subjected to gel filtration on Sephadex G-50. Material of molecular weight (M_r) below 1500 was disregarded.

3. Sephadex G-100 chromatography: Incubation medium (0.5 ml) was applied directly to a Sephadex G-100 column (1.8 × 70 cm) equilibrated with 0.2 M pyridine-acetate buffer (pH 5.0) and eluted at a flow rate of 10 ml/hr. Material of M_r below 4000 was disregarded.

4. TCA precipitation followed by lipid extraction, papain treatment, and Sephadex G-50 chromatography: The washed pellet from Procedure 1 was extracted with chloroform:methanol (2:1, by volume). Chloroform:methanol:water (10:10:3, by volume) and treated with papain as described from the cellular pellet [11]. Material of M_r below 1500 resulting from fractionation on Sephadex G-50 was disregarded.

Proteolytic Enzyme Treatment of Keratinocytes

After washing the cells 3 times with 0.5-ml portions of TC199 medium without serum, the keratinocytes were suspended in 200 μl TC199 medium (pH 7.2). Normally 100 μl 0.025% trypsin was added, but in some experiments this was replaced by 100 μl 0.125% trypsin or 100 μl 0.25% papain in the presence of cysteine (2 mg/ml). Control incubations contained TC199 medium instead of enzyme. After incubation for 5 min at 37°C with shaking, the tubes were cooled to 0° and 200 μl ice-cold TC199 medium was added. The cell suspension was centrifuged and the pellet washed with 500 μl TC199 medium. Supernatant and washing were combined and TCA-insoluble material was isolated, followed by extraction with chloroform-methanol as described for cellular material [11]. The remaining residue was digested with papain and the solubilized glycopeptides were fractionated by gel filtration on Sephadex G-50 [11]. In some experiments a direct papain digestion without TCA precipitation and chloroform-methanol extraction was performed on the material released by trypsin.

Calculations

All calculations of percentage distribution were based on the summated radioactivity from fractions A–C (i.e., material of $M_r < 1500$ was disregarded).

Percentage shed glycoprotein was estimated as:

$$\frac{\text{cpm shed}}{\text{cpm cellular} + \text{cpm shed}} \times 100\%$$

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Abbreviations:
M_r: relative molecular mass
TCA: trichloroacetic acid

and trypsin-released material as:

$$\frac{\text{cpm released}}{\text{cpm cellular + cpm released}} \times 100\%$$

RESULTS

Shedding of Labeled Glycoconjugates

Determination of the amount and the nature of radioactive material "shed" into the medium by keratinocytes was hampered by several factors, especially the extensive nonspecific binding of labeled sugars to macromolecules present in the serum [14,15]. Under the conditions of our experiments this amounted to 0.3% of the total added radioactivity using [^{3}H]-fucose. For this reason we have compared 4 different isolation procedures. Method 1 (precipitation of glycoconjugates with TCA) gave apparent values of conjugated sugar about 4 times higher than Methods 2, 3, or 4, even after correction for nonspecific binding. Using Method 4 all the nonspecifically bound sugar was extracted into chloroform-methanol as appears from incubations of TC199 medium supplemented with calf serum and labeled fucose but without cells. The nonspecifically bound sugar (present in the column volume using Methods 2 and 3) was absent in the column volume using Method 4; this was therefore selected for further experiments.

Calculated as percentage of total fucose-labeled glycoproteins, the shed material amounted to 10 ± 2, 18 ± 3, and 29 ± 7 (mean ± S.E., 5 experiments each) for normal, psoriatic uninvolved, and psoriatic lesion, respectively; only the values for normal and psoriatic lesion differ significantly from each other (Wilcoxon ranking test, $p < 0.05$). The corresponding elution profiles for shed glycoconjugates, as obtained by gel filtration on Sephadex G-50, are given in Fig 1. Nearly all radioactivity appears to be eluted in the *C* fraction, little difference being found between material derived from normal, psoriatic uninvolved, and psoriatic lesion (*C/B* ratios 6.2 ± 0.9, 7.8 ± 1.1, and 6.6 ± 1.3, respectively).

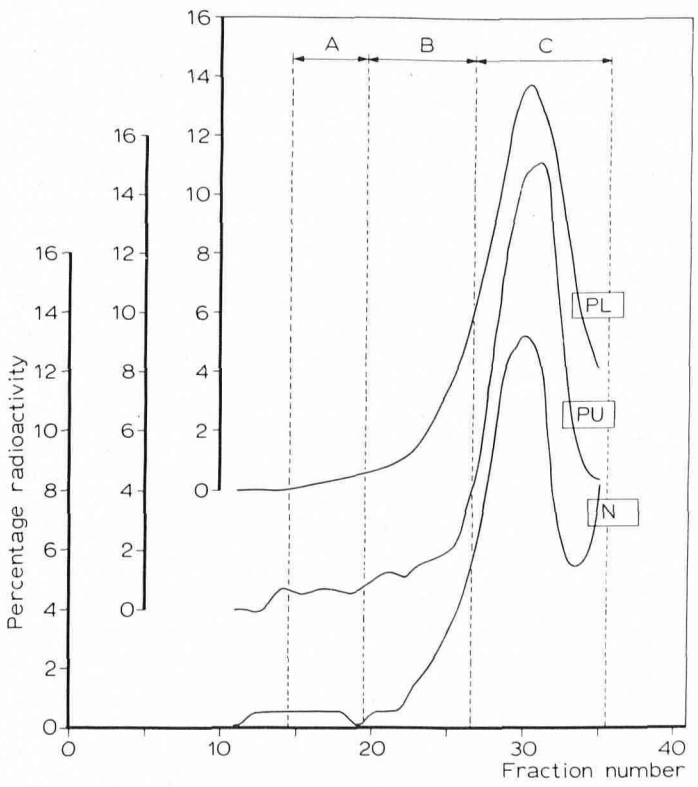

FIG 1. Elution profiles of shed fucose-labeled glycopeptides on Sephadex G-50. *N* = normal epidermis, *PU* = uninvolved epidermis from psoriatic patients, *PL* = psoriatic lesion. For subdivision into *A–C* (here taken as 100%) see Roelfzema et al [11].

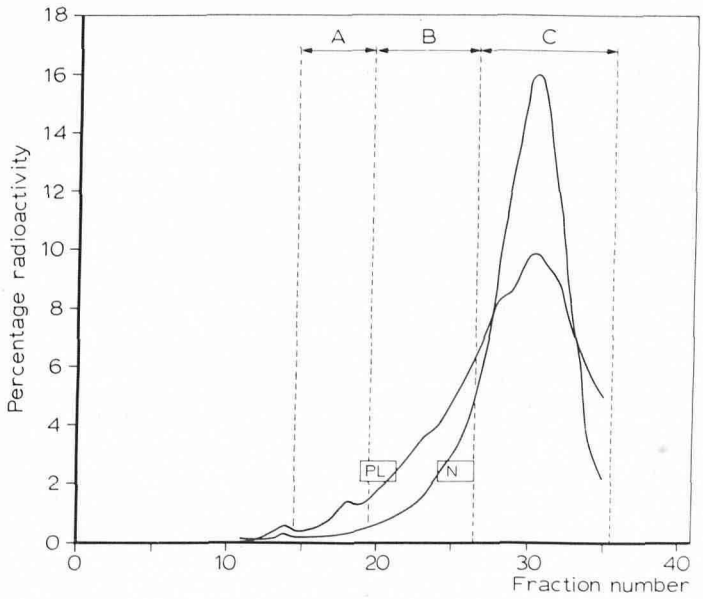

FIG 2. Elution profiles of tryptic fucose-labeled glycopeptides on Sephadex G-50. For symbols see Fig 1.

Proteolytic Enzyme Treatment of Keratinocytes

Alternative procedures used to release glycoconjugates from the cell surface of normal keratinocytes (incubation with a 5 times higher concentration of trypsin or employing papain instead of the 0.025% trypsin solution) led, qualitatively and quantitatively, to the same results as our standard method. Even a direct papain digestion of the trypsinate, omitting TCA precipitation and lipid extraction, produces a similar elution profile when chromatographed on Sephadex G-50. Interestingly, the M_r distribution of fucose-labeled glycopeptides from trypsinates of normal keratinocytes (Fig 2, *curve N*) is identical to that of the shed material, the great majority of the radioactivity appearing in the *C* fraction.

Quantification of the material released by proteolysis gave figures of 7 ± 1 and 11 ± 2 (mean ± S.E. for 6 experiments) for percentages of the total labeled glycoproteins from keratinocytes from healthy epidermis and psoriatic lesions, respectively; these are not significantly different ($p > 0.1$, Wilcoxon rank test). However, the M_r profile for the psoriatic material (Fig 2, *curve PL*) is quite distinct from the normal pattern, the *C/B* ratio being reduced from 6.0 ± 0.2 to 2.7 ± 0.2 ($p < 0.01$).

DISCUSSION

Comparison of 4 alternative procedures for determining the cell-surface glycoconjugates shed into the medium illustrates the pitfalls in obtaining a reliable estimate of this parameter. First, as pointed out above, a correction must be made for sugar which is nonspecifically bound to macromolecular components of the incubation medium. Unfortunately (especially using low cell densities or cells of low metabolic activity), this blank may be a very large proportion of the total labeled glycoconjugate. Second, gel filtration of papain digests of the chloroform-methanol extraction TCA precipitate (Method 4) made it clear that much of this fraction had a $M_r < 1500$ and was therefore not shed surface glycoconjugate but presumably cytoplasmic intermediates, which "leak" into the medium. This was confirmed by the fact that Method 2 (omitting TCA precipitation and chloroform-methanol extraction) and Method 3 (direct gel infiltration of medium on G-100, taking $M_r < 4000$ as "nonconjugated" sugar) both gave quantitatively similar results to Method 4. Method 4 was selected for routine analysis since the relatively low blank resulted in much greater reproducibility.

The increased shedding of fucose-labeled glycoproteins for

keratinocytes derived from psoriatic lesion compared to normal and psoriatic uninvolved skin corresponds well with the increased turnover rate of fucosylated glycoproteins previously reported [10]. The sharply defined M_r of carbohydrate moieties of the shed glycoproteins (Fig 1), in contrast to the relatively complex cellular glycoprotein composition [11], further suggests that only one particular class of fucosylated glycoproteins show a rapid turnover. Judged by the *C/B* ratios of shed glycopeptides derived from normal, psoriatic lesion, and psoriatic uninvolved skin (6.2, 6.6, and 7.8, respectively) and the *C/B* ratios of concanavalin A-bound cellular glycopeptides (6.2, 3.5, and 7.3, respectively [12]), this class is composed of "biantennary" structures. A similar finding has been reported by Warren et al [1] who showed that glycoproteins shed into the medium by either control or transformed hamster cells contain predominantly glycopeptides having a high affinity for concanavalin A-Sepharose. The similar elution profiles obtained for glycopeptides derived from the trypsinate and the shed material from normal keratinocytes suggest that the glycoproteins shed into the medium are derived from the cell surface [16].

Trypsinization or papain treatment of normal and psoriatic keratinocytes appears to release only a small portion (7% and 11%, respectively) of the cellular-labeled glycoproteins. By comparison, figures of 19–30% have been reported for cells of nonepithelial origin [17,18]. It is likely that the cell surface glycoprotein of keratinocytes is relatively "inaccessible" to the proteolytic enzyme, since the use of autoradiographic techniques indicates that 79% and 44% of the fucose-containing glycoproteins are in fact located on the plasma membrane of normal and psoriatic keratinocytes, respectively [19]. When the M_r distribution of glycopeptides derived from trypsinates is compared with total cellular glycopeptides, it appears that the cell surfaces for both types of keratinocytes contain relatively increased proportions of low-M_r glycopeptides. For fibroblasts, Muramatsu et al [20] reported that the carbohydrate structures for tryptic glycopeptides were similar to the glycopeptides from the remaining cellular material.

Increased proportions of high-M_r fucose-labeled glycopeptides appear to be present in the trypsinates of psoriatic keratinocytes compared to normal (Fig 2), demonstrating that previously reported differences in the overall glycoprotein composition [11] also occur in their cell surfaces. Because the high-M_r fucose-labeled glycopeptides (*B fraction*) are nearly absent in the shed material, this may point to the appearance of a particular class of glycoproteins (with a relatively low content of biantennary structures) in the plasma membrane of psoriatic keratinocytes. The observed differences in turnover and composition of cell surface glycoproteins between normal and psoriatic keratinocytes strengthen the hypothesis that altered glycoproteins are involved in the loss of growth control of psoriatic epidermis.

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